P:\KL\LEUK016

RESEARCH, DEVELOPMENT AND MARKETING AGREEMENT

Research, Development and Marketing Agreement, dated as of between LEUKOSITE, INC., a Delaware corporation ("LeukoSite"), located at 800 Huntington Avenue, Boston, Massachusetts 02115, and WARNER-LAMBERT COMPANY, a Delaware corporation ("Warner"), located at 201 Tabor Road, Morris Plains, New Jersey 07950.

WITNESSETH:

WHEREAS, LeukoSite and Warner each has certain expertise in the discovery and development of compounds that inhibit the action of MCP-1 (the "Field"); and

WHEREAS, Warner and LeukoSite each wishes to enter into a collaborative effort to share such expertise, to develop new expertise in the Field, to research together potential applications thereof and, if successful, to market certain of such applications (the "Collaboration");

NOW, THEREFORE, in consideration of the foregoing premises and the mutual promises, covenants and conditions contained herein, LeukoSite and Warner agree as follows:

ARTICLE A

DEFINITIONS

The following capitalized terms shall have the following meanings for purposes of this Agreement:

"Affiliate" shall mean any corporation, association or other entity which directly or indirectly controls, is controlled by or is under common control with the party in question.

12.17 In the event that there is a conflict between the text of this Agreement and Exhibit 1, the text of this Agreement shall control.

IN WITNESS WHEREOF, the parties hereto have caused this Agreement to be ~~executed~~ Signed by their duly authorized officers to be effective as of the date first above written.

LEUKOSITE, INC.

By: _____

Name:

Title:

WARNER-LAMBERT COMPANY

By: _____

Name:

Title:

EXHIBIT
tabbies
p. A-2

12.17 In the event that there is a conflict between the text of this Agreement and Exhibit 1, the text of this Agreement shall control.

IN WITNESS WHEREOF, the parties hereto have caused this Agreement to be ~~executed~~ signed by their duly authorized officers as of the date first above written. (to be effective)

LEUKOSITE, INC.

By:_____

Name:

Title:

Date:

WARNER-LAMBERT COMPANY

By:_____

Name: L.J.R. de Vink

Title: President & COO

Date:

EXHIBIT
tabbies
p. A-3

EXHIBIT I

LeukoSite, Inc.:Parke-Davis Collaborative
Research and Discovery Program

Overview of collaborative research plan for the discovery of an MCP-1 antagonist or an MCP-1 triggered signal transduction inhibitor.

Overall Objective

The overall objective of the collaborative research and discovery program is to identify a small-molecule, synthetic antagonist that inhibits the action of MCP-1. The basis of this program will be the screening of chemical libraries using *in vitro* receptor-ligand binding assays and MCP-1 triggered, cell-based signal transduction assays. From this effort it is expected that structures (preleads) will be identified and that from these structures, chemical analogs will be synthesized and undergo evaluation in *in vitro* and *in vivo* assays as part of a structure-activity relationship program. Compound(s) with optimized pharmacological parameters and pharmaceutical properties [as defined by the Research and Management Committees] will be recommended as candidates for preclinical development (ie, lead compounds). If a compound is selected for development, LeukoSite and Parke Davis may jointly develop and commercialize the drug (as per the terms defined in the Research, Development and Licensing Agreement). In addition, LeukoSite and Parke-Davis will exchange all reagents and information necessary for both companies to carry out the research plan and understand the details of activities conducted at the other company relating to the MCP-1 inhibitor program.

The Process

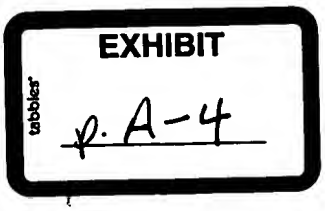
The MCP-1 Antagonist Discovery Program will proceed in two stages:

Stage 1

During this stage 1° and 2° assays will be developed and validated and compound libraries screened. In addition, proof of concept studies will be performed in a number of experimental animal studies with a variety of tools, including blocking mAbs. At the conclusion of Stage 1:

- The program will move on to Stage 2 if pre-lead structures have been identified (as defined by the Research Committee) and animal models using mAbs and other *in vivo* studies continue to support the hypothesis that blockade of MCP-1 function would have significant anti-inflammatory and -autoimmune activity.

  If no pre-lead compounds are identified, then the MCP-1 Program would be terminated (as per the Agreement).



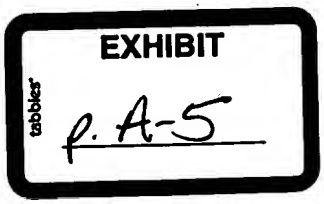
- If there continued to be interest in additional chemokine receptors, than an alternate target could be selected (as per the agreement). Activities similar to those in Stage 1 would then take place.

- If Parke-Davis decided to curtail their interest in chemokines and chemokine receptors then the collaboration would be terminated.

Stage 2

During this stage, medicinal chemistry would be undertaken around pre-lead compound structures. Structure activity relationships would be defined and selected compounds would undergo *in vivo* pharmacologic evaluation. Compound(s) meeting a predefined set of pharmacological potency/selectivity and pharmaceutical proprietary criteria would be selected for recommendation to the Management Committee as lead compound(s) for development (as per the Agreement).

The following is a more detailed summary of the activities and milestones contained in Stage 1 of the proposed joint MCP-1 antagonist program.



Outline of LeukoSite/Parke-Davis Collaborative Program
for the Discovery of Lead Compounds as Antagonists of MCP-1

I. Plan for Year 1

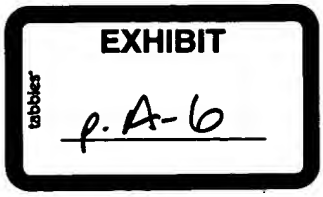
Aim

- Develop MCP-1 screening assays for ligand binding and signal transduction.
- Screen libraries of chemical compounds.
- Identify active compounds.
- Initiate medicinal chemistry SAR.
- Provide proof of principle in at least one animal model.

Actions	Responsibility
• Provide MCP-1 for assays and structural studies	Joint
• Express MCP-1 receptors A,B	LeukoSite
• Develop stably expressing cell lines	LeukoSite
• Develop blocking antibody to MCP-1	Joint
• Develop blocking antibodies to receptor	LeukoSite
• Develop various chemotaxis assays	LeukoSite

• Structure function studies
  • Chemokine
  • Receptor
	Joint
	LeukoSite

• Develop ligand binding assay	LeukoSite
• Develop scintillation proximity assay	Parke-Davis
• Develop signal transduction assay	LeukoSite
• Screening	Joint
• Initiate active compound optimization	Joint
• Demonstrate proof of principle	Joint



II. Rationale and Brief Description of Year 1 Research and Discovery Activities

- Chemokine

MCP-1 is currently being purchased with a bulk discount from Peprotech. LeukoSite also has the full length cDNA and is working out optimum conditions for bacterial expression. Depending on the outcome LeukoSite may have to consider non-bacterial expression systems. Availability of mg quantities of chemokine is important for other projects and to provide sufficient material for solution NMR and crystal structure studies LeukoSite is proposing as a joint effort with Parke-Davis. In addition MCP-1 is needed for routine chemotaxis work. NEN now sells ^{125}I-MCP-1, which may fill short term needs in ligand binding. Parke-Davis will assume responsibility for production of mg quantities of MCP-1 for joint efforts.

- Receptors

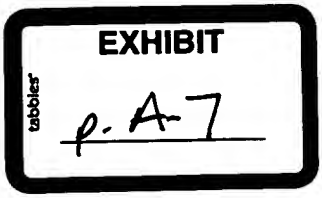
The MCP-1A and MCP-1B receptors are now available. They are critical for many aspects of this program. One of LeukoSite's earliest efforts will be to validate these receptors in in a variety of assays in different host contexts as in fact high affinity MCP-1 receptors. To date all published information bears solely on the induction of a Ca^{+2} flux in frog oocytes. LeukoSite has prepared full length constructs with flag DYKDDDDK and without the flag sequence (DYKDDDK) at the amino terminus. In addition, LeukoSite will transfect with constructs containing a leader (CD5) peptide sequence, an approach that has given good results in Eugene Butcher's lab. Validation of these receptors in LeukoSite's own hands will be accomplished by demonstrating in ligand binding studies that MCP-1 binds with high affinity (low nanomolar) and specificity as evidenced in competition binding assays.

- Develop stably expressing cell lines

Several host cell types are being used for making stable MCP-1R constructs, including L1, 2 cells, L cells and CHO cells. These must meet the several needs discussed below such as for ligand binding and signal transduction assays as well as for structure function studies in Dictyostelium and the generation of monoclonal antibodies.

- Produce blocking antibodies to MCP-1

LeukoSite has some monoclonals that are being evaluated for blockade and will be doing additional fusions to develop these antibodies. They will serve various needs including (possibly) proof of principle studies, ELISA development, immunohistology and various research needs. In addition LeukoSite and Parke-Davis will collaborate to prepare blocking polyclonal antibodies to human and rat MCP-1 for proof of principle studies in primates and rats.



- Produce blocking antibodies to MCP-1R

These antibodies will be especially important for proof of principle studies, provided we obtain appropriate species cross-reactivity. These antibodies will help with research objectives concerning the distribution and expression of the receptor on different cell lineages. Such distribution studies may provide important clues to potentially adverse reactions of antagonists as well as other potential therapeutic opportunities. Initially these will be raised against N terminal peptide fragments, and subsequently against murine cells expressing high levels of receptors.

- Bring on-line chemotaxis assays

These are essential to the core research program and for secondary screening efforts on leads that come out of our primary screens. Chemotaxis assays for research-scale efforts are operating for mononuclear cells and neutrophils. Additional efforts are underway to optimize the use of endothelial cell lines to replace umbilical vein endothelial cells and to provide receptor transfectants that will migrate in standard chemotaxis assays. Longer-term studies may lead to development of high throughput chemotaxis assays in 96 well formats.

- Ligand binding assay

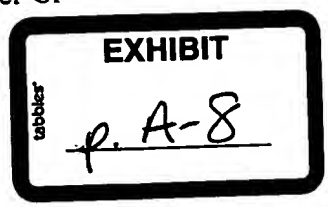
This assay will use radiolabeled MCP-1 for binding to cell membranes. This will be one of two primary high throughput screens. Receptor will be derived from stable A or B transfectants which express high levels at the cell surface. Joint efforts with Parke-Davis will be directed to development of a scintillation proximity assay.

- Develop signal transduction assay

This will be the second primary screen. LeukoSite proposes to couple ligand binding to receptor with the regulation of integrin affinity and, thereby, adhesion to an appropriate ligand such as VCAM or fibronectin. Efforts will be directed to use of appropriate human cell lines. Additional experiments are in the planning/feasibility stage to assess the development of an appropriate reporter gene construct in Dictyostelium. Such an assay would have to use Dictyostelium pathways with human receptors and G proteins.

- Structure/function studies

Research efforts will begin to identify critical regions of ligand-receptor interaction. Much of the receptor mutagenesis studies will be carried out in Dictyostelium because the manipulability of these cells. Validation of results from Dictyostelium will be done in mammalian lines. Structural studies of MCP-1 itself may provide antagonist peptide leads. Several approaches in addition to NMR/crystal studies will be taken, including mutagenesis, domain "swapping" and phage displays of chemokine. In addition LeukoSite's panel of antibodies may give clues to functional domains.



- Screening

The primary ligand binding and signal transduction assays will be used to screen compounds. The primary assays will be transferred to Parke-Davis for the screening of the Parke-Davis compound collection. LeukoSite has begun to acquire a compound collection in which it aims to have ~ 20,000 chemicals within the next 12 months.

In keeping with the objective of discovering an anti-MCP-1 receptor antagonist or MCP-1-triggered signal transduction inhibitor with appropriate specificity and without undue broad inhibitory activities, certain secondary screening assays will be performed in Phase I. The primary aim of Stage I secondary assays will be to demonstrate specificity for cells expressing the MCP-1 receptor and absence of activity for cells lacking the receptor. Due to concerns about unduly compromising host bactericidal activity, compounds identified in either primary screen will be evaluated for activity on the IL-8 and C5a mediated chemotactic responses of neutrophils. In addition, LeukoSite will evaluate compounds for inhibitory activity in chemotaxis assays of T cells and monocytes with other CC chemokines as they become available. Screens will be constructed so as to allow evaluation of distinct effects on cell migration and viability. It is anticipated that a more thorough analysis of compound effects will take place in Stage II. Lastly, compounds of several distinct molecular classes, if available, will be screened. This will give us structural criteria which in addition to specificity and toxicity criteria will serve as a basis for designation of a Stage I lead compound.

Pharmacologic Characterization of Compounds Identified in Primary Screens and Further Evaluated in Secondary Screens During Stage I

1° Assays	Property
• MCP-1 ligand binding	Receptor antagonist
• MCP-1 cell-based assay	MCP-1 triggered signal transduction inhibitor
2° Assays	
• T cell/monocyte chemotaxis to MCP-1	Chemotaxis antagonist in physiological setting *in vitro*
• T cell/monocyte chemotaxis to other CC chemokines	Fine specificity of antagonist activity
Neutrophil chemotaxis to IL8	Effect of antagonist on CXC receptors IL8A and IL8B
Neutrophil chemotaxis to C5a	Effect on non-chemokine G protein coupled receptor

- Viability of dividing and/or chemotaxing MCP-1R bearing cells — Cellular "therapeutic" index

The selection criteria for potency and degrees of specificity will evolve as the screening process begins. The Research Committee will define the desired profile for recommending candidates to the Management Committee for approval as Stage II lead compounds.

- Initiate SAR studies

A goal of this project will be to identify an active structure with potency in the high nanomolar to low micromolar range which can undergo SAR efforts. It is likely that a preliminary SAR may evolve following evaluation of compound structures from the primary screens. Limited synthetic chemistry may be performed on selected compounds in Stage I to facilitate selection of Stage II lead compounds.

- Demonstrate proof of principle

A key objective in the first year will be to target the chemokine with neutralizing antibodies in at least one inflammation model. First attempts will be with rabbit anti-rat MCP-1 in arthritis (Parke-Davis) and delayed-type hypersensitivity (LeukoSite).

III. Forecast of LeukoSite scientific staff to be allocated to various activities within the first year of the collaborative program.

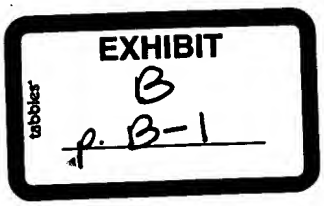
Activity	Person years
Establish ligand binding assay	0.5
Establish signal transduction assay	2
Monoclonal antibody efforts	2
Proof of principle (at LeukoSite)	1
Structure function studies	1
Chemokine and receptor expression	1
Chemotaxis efforts	1.5
Total	9

IV. Time lines for Activities

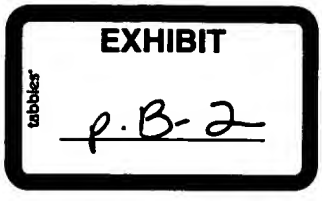
Figures 2 and 3 show the various activities to be undertaken in year one of the collaboration and the projected start/finish date for each. These timings are best approximations based on current assumptions of A) an start date for the collaborative agreement, B) the level of resources and support to be given to the program and C) best case scenarios with respect to the outcome of the experimental plan.

Greg LaRosa
Senior Scientist, Immunology
Goals & Objectives

	WT	Target for completion
A. MCP-1 Receptor Program		
1. Coordinate activities of LeukoSite staff on MCP-1 program work, including: Lijun, receptor expression in Dictyostilium; Heidi, chemotaxis assays; Nasim, antibody generation; Ling and myself, ligand binding, and cell line and assay development; ET group, *in vivo* model and antibody assays.	5%	ongoing
2. Maintain contact with Parke-Davis group via Steven Hunt to foster open communication and to develop collaboration.	5%	ongoing
3. Investigate the expression of various MCP-1R constructs, including the native receptors, N-terminal signal peptide, chimeras with other chemokine receptors, and other alterations, as well as other vector/promoters.	25%	Q1,
4. Establish system for the expression of the cloned MCP-1 receptors in stable transfected cell lines: 1. CHODG44 or HEK293 for source of membranes with high density of receptor 2. L1-2 and/or 300.19 for immunogen to generate monoclonals to native receptor 3. HUT78, U937, THP-1, and/or Jurkat for establishment of MCP-1 responsive signal transduction assay	25%	Q1,
5. Characterize the expression in the above cell lines using: 1. Ligand binding 2. Immunochemistry 3. Chemotaxis 4. Ca^{2+}-flux	25%	Q2,



	WT	Target for completion
6. Develop signal transduction assay for MCP-1 receptor activity that can be formatted for 96-well plate, high-throughput screen. Test feasibility of the following: 1. Adhesion 2. Chemotaxis 3. Ca^{2+}-flux (?)	25%	Q2,
7. Establish methods for MCP-1 receptor membrane ligand binding assay amenable to high-throughput screen.	15%	Q4
8. Assess the activity of currently available MCP-1 and MCP-1 receptor-directed antibodies: 1. Cell surface staining 2. Western blot 3. Affect on binding, chemotaxis, ect.	15%	Q4
9. Produce additional receptor MCP-1 receptor-directed antibodies using murine cell line MCP-1 receptor stable transfectants.	15%	Q2,
10. Establish and moniter collaborations with Wayne Smith, Israel Charro, and Barrett Rollins.	5%	ongoing



Greg LaRosa

PERFORMANCE PLANNING

I. Statement of Objectives

A specific statement of each of the major objectives the employee is expected to achieve.

Objective	WT(%)	Target Date	Status
1. Begin to coordinate activities of LeukoSite study on MCP-1 program & maintain contact with S. Hunt at Parke-Davis to develop collaboration	5	ongoing	have established frequent contact with S. Hunt & J. Marki @ Parke-Davis
2. Investigate expression of various MCP-1R constructs to determine best form to go into stable transfectants	25	Q1	have gotten good expression of several constructs; have made native receptor
3. Establish methods for membrane ligand binding assay with MCP-1 & THP cell membranes	25		have been successful in getting membrane binding; working on optimizing & making more reproducible
4. Establish signal transduction assay for MCP-1 activity. Test various cell lines for endogenous MCP-1 response & adhesion. May require the generation of stable transfected cell lines	25	end Q1	Screening HUT78 transfectants cells for receptor expression
5. Assess activity of currently available MCP-1 & MCP1R mAbs	15	Q2	Tested 5A11 & 5E11 for binding to transient transfectants & have been binding. Purified 5A11 IgG

Footnotes:

Specific comments on objective:

1.
2.
3.
4.

EXHIBIT tabbies p. B-3

I certify that this report has been discussed with me. I understand that my signature does not necessarily indicate agreement.

Employee Signature Date

Supervisor's Signature Date

Title Senior Scientist

Title Chemist Manager

Social Security Number ———

TITLE HS 105 MCP-1 Receptor fusion Project No. Book No. 175 101

From Page No. Feb 16/97

HS 105 MCP-1 R fusion

[9 C57 Black mouse was immunized as follows

11/18/96 3Gy NBA & Hilongan treated cells 2×10^7 I.P.

11/26/96 3Gy NBA & Hilongan treated cell 2×10^7 I.P.

12/11/96 3Gy NBA & Hilongan treated cell 2×10^7 I.P.

12/31/96 3Gy NBA treated cell 2×10^7 I.P.

1/10/97 3Gy NBA treated cell 2×10^7 I.P.

1/24/97 3Gy NBA treated cell 2×10^7 I.P.

2/13/97 3Gy NBA treated I.V. 2×10^7 cell

Cell count:

Spleen cell — SP2/0

1.4×10^8 total cell — 1.4×10^8 also — used 1/5th of them.

Cell count was done by Dulce

Fused as per usual using 2 ml of 50% PEG (1:4)

& resuspended into 200 ml of HAT media Distributed

into 10-11-96 well plate 200 ml/well To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	3/14/97	Recorded by	2/18/97

EXHIBIT C

tabbies

TITLE HS 110 PPD#2 fusion Project No. Book No. 175

163

From Page No. May 1/97

HS 110 PPD#2 fusion

1 F CS7 Blade name was changed as follows:

11/8/96	B-10 CCR2.300 19	Milonycin	Incubated 1.5×10^7 I.P.
11/15/96	Milonycin Incubated	B-10 cell	1.5×10^7 I.P.
11/22/96	Milonycin Incubated	B-10 cell	1×10^7 I.P.
12/12/96	Cell (B-10)	1×10^7	I.P.
12/27/96	Cell	B-10	1×10^7 I.P.
1/10/97	Cell	B-10	1×10^7 I.P.
1/23/97	Cell	B-10	1×10^7 I.P.
2/13/97	Cell	B-10	1×10^7 I.P.
2/20/97	Cell	I.P.	1×10^7
3/6/97	PPD #2 + CFA	20 mg	I.P.
4/10/97	PPD #2	+ IFA 30 mg	I.P.
4/28/97	PPD #2	100 mg	I.V. (Shin.)

Cell count:

SP2/0	$47 \times 2 \times 10^4$/ml 2.82×10^7	30 ml	fused as per usual & distribute into 10-11 plates 20 ml/well.
Spleen	110×10^5 3.3×10^8	30 ml	

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	5/29/97	[signature] Recorded by	5/1/97

EXHIBIT tabbies D

LS 119 PPD #2 fusion 220

Aug 15/97

1 C57 ♀ black mouse was immunized as follow

11/8/96 B-10 CCR2 300-19 Hibonyin Inoled cell I.P 1×10^7

11/15/96 B-10 CCR2 300-19 Hibonyin Inoled cell I.P 1×10^7

11/26/96 B-10 CCR2 300-19 Hibonyin Inoled cell I.P 1×10^7

12/2/96 B-10 CCR2 300-19 cell 1×10^7 I.P.

12/27/96 B-10 CCR2 300-19 cell 2×10^7 I.P.

1/20/97 B-10 CCR2 300-19 cell 2×10^7 I.P.

1/23/97 B-10 CCR2 300-19 cell 1×10^7 I.P.

2/13/97 B-10 CCR2 300-19 cell 1.5×10^7 I.P.

2/20/97 B-10 CCR2 300-19 cell 2×10^7 I.P.

3/6/97 PPD #2 +CFA 20 ug I.P.

4/10/97 PPD #2 +IFA 30 ug I.P.

8/12/97 PPD #2 I.V. 50 ug (by Shu).

cell count:

Spleen

73×10^5/ml 30 ml

2.2×10^8

LN26

$66\times2\times10^4$

1.32×10^5/ml 15 ml

1.98×10^7

Fused as per usual & resuspended into 200 ml of HAT media (10-11 plates)

Witnessed & Understood by me:	Date	Invented by	Date
	12-22-97		8/18/98
		Recorded by	

EXHIBIT

tabbies

E

From Page No. 42

Friday, September 5th 1997

DNA: CCR2b. DEF3 ScaI } used 25 μg/transfection } 20.8 μl (1.2 3/4)
CCR2b. PEFIRES XhoI } } 16.7 μl (1.5 3/4)

Two electroporation protocols followed, The Nancy Ruffing method and the Amy Reinhart Method 50×10^6 cells used for each

	Nancy Ruffing Method	Amy Reinhart Method
Buffer	HBSS (no phenol red)	Special electroporation buffer (see results book eight for recipe)
cells washed?	yes	no
machine settings	250 volts, 960 μFd	450 Volts, 960 μFd
sit for 10" afterwards?	yes	no
tRNA?	yes	no
time constants:	DEF3 12.5 PERIRES 12.5	DEF3 13.3 IRES 13

Cells transfered to 15 ml L1/2 medium (no G418) and grown over the weekend.

Monday, September 8th 1997

Cells centrifuged out of medium and resuspended in G418 medium

To Page No. 56

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
Suzanne W. Densmore	3/13/98	Recorded by Amy L. Reinhart	9/5/97

EXHIBIT F p. F-1

10x **1.2M L-Glutamic Acid** G-1149

MW = 185.2

$185.2 \frac{g}{mole} * 1.2 \frac{mole}{L} * .05L = \boxed{11.11g}$

2x **10mM ATP**

MW = 551.1

$551.1 \frac{g}{mole} * 0.01 \frac{mole}{L} * 0.05 L = \boxed{0.2755g}$

10x **70mM Mg Acetate**

MW = 214.46

$214.46 \frac{g}{mole} * .07 \frac{mole}{L} * 0.05L = \boxed{0.7506g}$

10x **43mM Glucose**

MW = 180.2

$180.2 \frac{g}{mole} * .043 \frac{mole}{L} * 0.05L = \boxed{0.38743g}$

10x **170mM K Pipes, pH 6.9**

MW = 378.5

$378.5 \frac{g}{mole} * 0.170 \text{ moles} * 0.05L = \boxed{3.22g}$

10x **10mM EGTA**

MW = 380.4

$380.4 \frac{g}{mole} * 0.01 \frac{mole}{L} * 0.05L = \boxed{0.1902g}$

LEUKOSITE INC.

Date: 09/05/97
Time: 02:20

Oleic Acid
ReadSamples | Method | SaveClear | Print | Quit

Results file: A:\WORK_RES

Method name: A:\DEFAULT

Assay type: General Ratio and Concentration
Formula setup: VIEW
Sampling device: None
Read average time: 0.50 sec

Units: ug/ml
Background Correction: [No]
Concentration: [No]
Peak Pick: [No]

Sample ID	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm
2IRESXHO	0.3013	0.1779	1.6937	0.5904 1.5 ug/ml
2DEF3SCA	0.2387	0.1583	1.5076	0.6633 1.2 ug/ml

LEUKOSITE INC

Lab Record
Book number 213
Page number 45

EXHIBIT
tabbies
p.F-3

56 Project No. [redacted] Book No. 213 TITLE Experiment [redacted]48/Chemotaxis/FACS Sorting

From Page No. 45

Wednesday, September 24th 1997

chemotaxis selection of: CCR2b. DEF3 (A)
CCR2b. PEFIRES (A)
CCR2b. DEF3
Note that CCR2b. PEFIRES(N) died off. Untx L1/2 used as negative control

Conditions: 3.0 micron B-D plates used (24 well) Lot# 902126
1 nM mcp-1 in 600 µl RPMI + 0.5% BSA
1×10^6 cells in 100 µl RPMI + 0.5% BSA

Cells allowed to chemotax for Four hours and twenty minutes.

MCP-1 1:100 in ddH_2O 117 µM → 1.17 µM = 1170 nM 2 µl → 200 µl
dilute this 1:1,170 to get 1 nM 117 µl + 10 µl 0.2 µM filtered.

Friday, October 9th 1997 Amy L. Reinhart 10/9/97

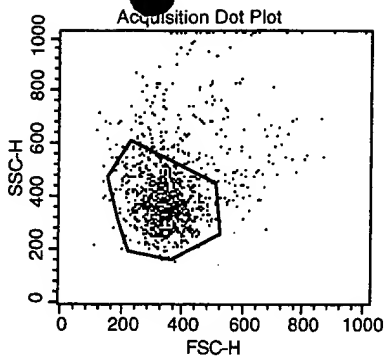
10^7 CCR2b. DEF3 stained with 5AII supernatant (50:50 supernatant PBS + Human serum) (filter sterilized) and 1:250 FITC Goat α mouse IgG (filter sterilized). Staining done in a volume of 5 ml. Sorted cells grown up starting from one well of a 24 well plate.

Tuesday, October 24th 1997 Amy L. Reinhart 10/24/97

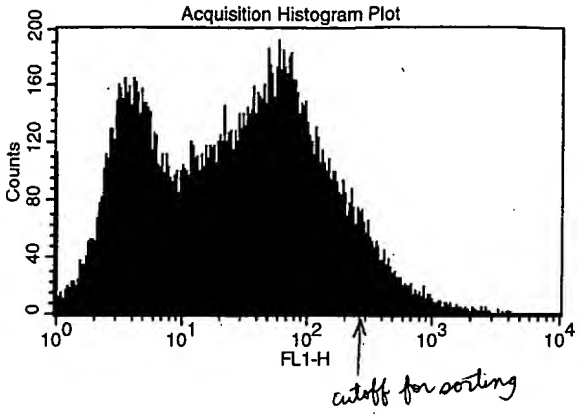
Sorted cells from October 9th, 1997, stained again using the same protocol and sorted.

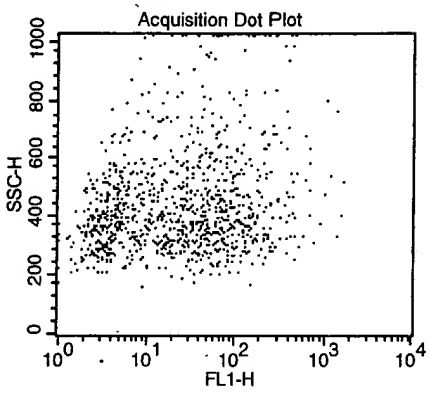
To Page No. ____

Witnessed & Understood by me,	Date	Invented by	Date
Suzanne W. Densmore	3/13/98	Recorded by Amy L. Reinhart	9/24/97



Unsorted CCR2b, DEF3





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Fust sort 10/9/97

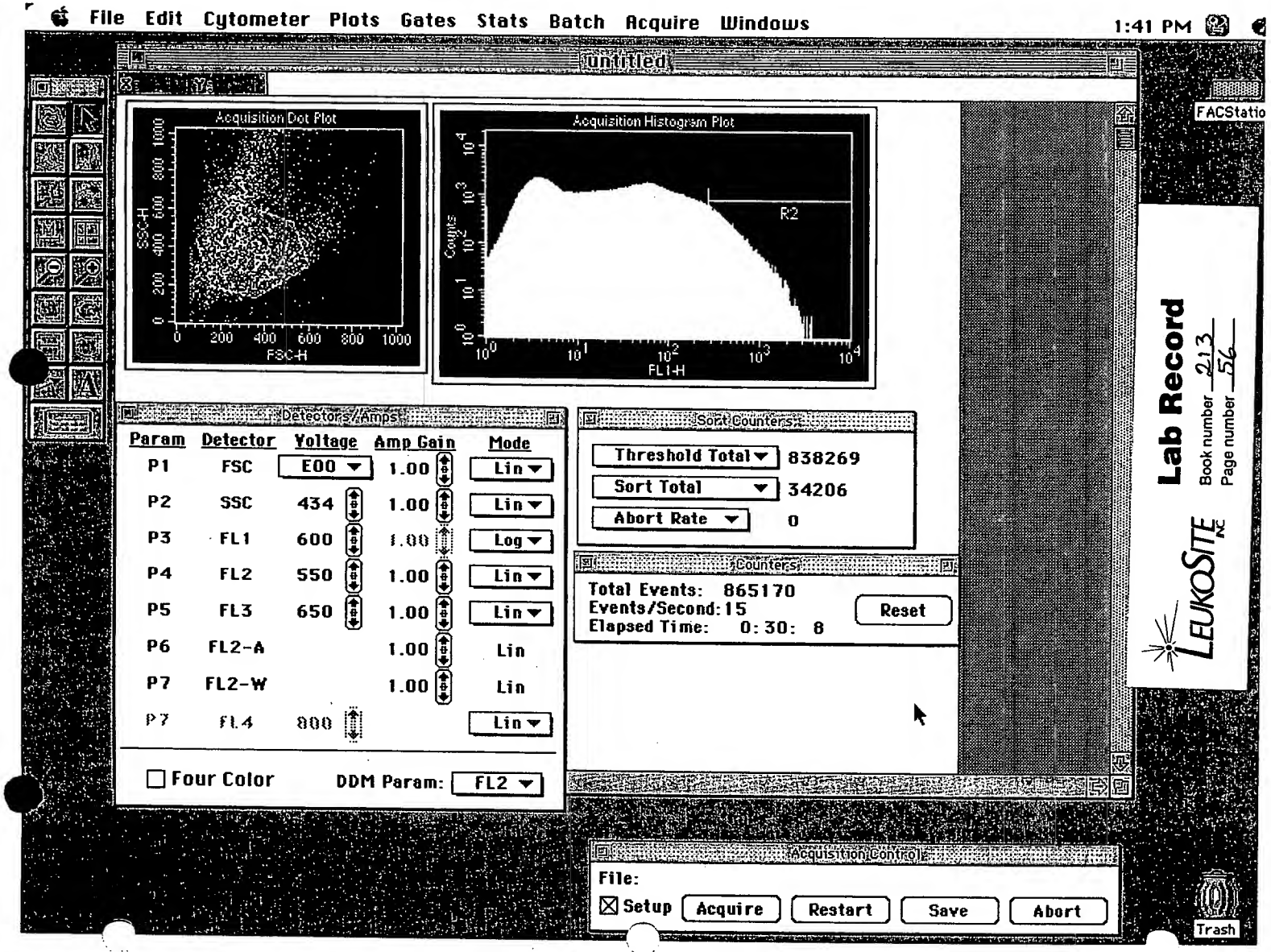

EXHIBIT
P.6.3
labbies

second sort 10/24/97

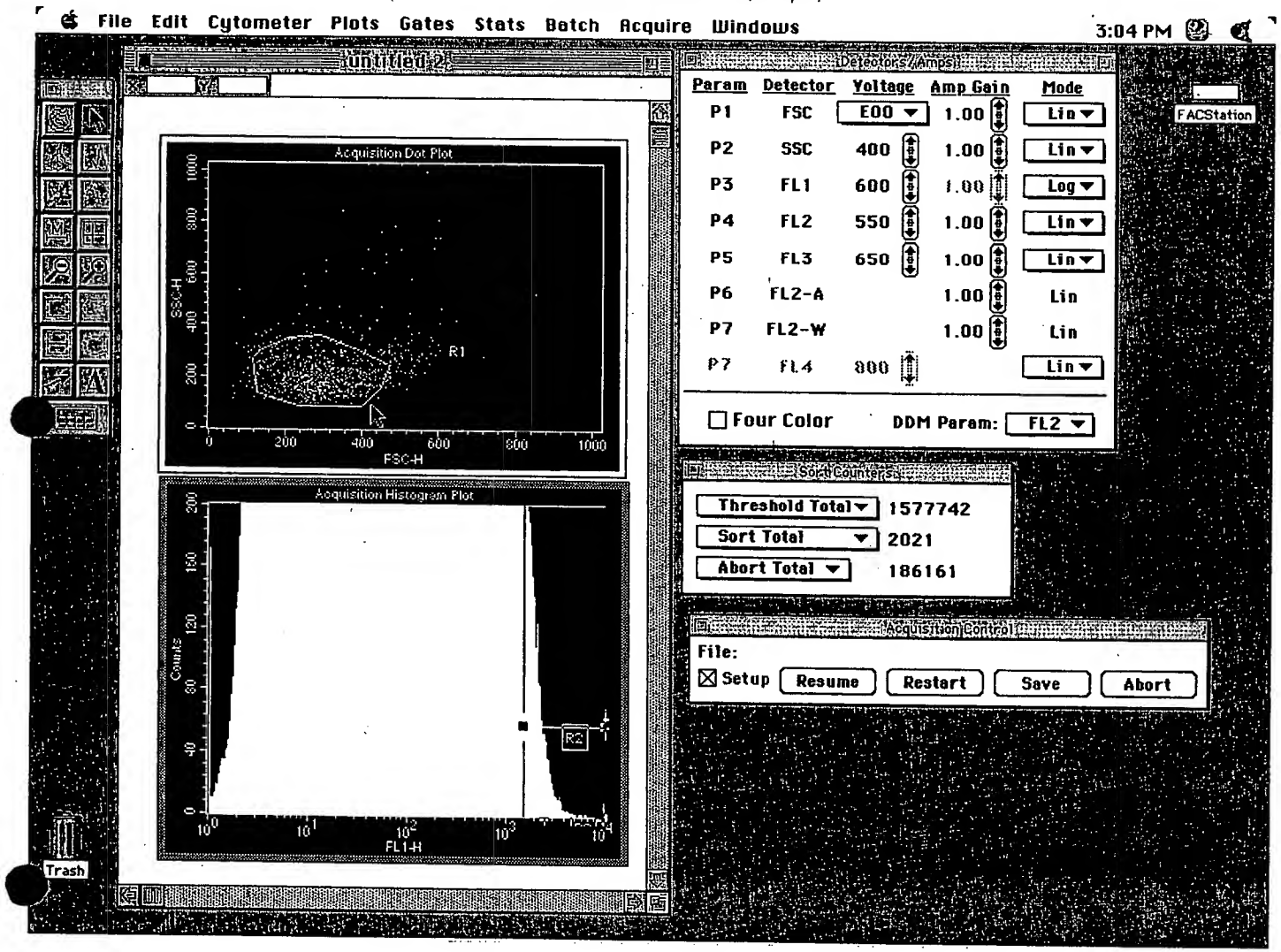

EXHIBIT 4-9-4 tabbies

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Book Number 213

Page Number 56

Project No. [redacted] kN .213 Experiment #278/

TITLE Preparation of [redacted]CR2b.DEF3 for injection

111

From Page No. 75

Wednesday, November 19th 1997

See p 75 (book 213) for protocol

240×10⁶ cells prepared. (Mitomycin C treatment)

Thursday, December 4th 1997 Amy L. Reinhart 12/4/97

Prepared 300×10⁶ cells. (Mitomycin C treatment) New bottle of mitomycin C.

~~Friday~~ Wednesday, December 10th 1997 Amy L Reinhart 12/10/97

One of the "new" mice has died.

Friday, December 19th 1997 Amy L. Reinhart 12/19/97

Another mouse developed a large tumor and was sacied,

Prepared 300×10⁶ cells (mitomycin C treated this, the fourth injection because of mice developing tumors.)
New bottle of mitomycin C.

Tuesday, January 6th 1998 Amy L. Reinhart 1/6/98

Seven (new) mice are left. Injected with 20×10⁶ CCR2b.DEF3 clone #60 per mouse I.P.

To Page No. 115

Witnessed & Understood by me,	Date	Invented by	Date
Suzanne Wensmire	3/20/98	Recorded by Amy L. Reinhart	11/19/97

EXHIBIT

tabbies

H

TITLE D132 CCR2 Jurkat Project No. Book No. 261 1

From Page No. Dec. 22/97

1 of CS7/Blade name was immuned as follows

11/8/96	B-10	CCR 26-300-19	Mitomycin cell I-P 1.5×10^7
11/15/96	B-10	CCR 26-300-19	Mitomycin cell I-P. 1.5×10^7
11/22/96	B-10	CCR 26-300-19	Mitomycin cell I-P 1×10^7
12/12/96	B-10	Cell	1×10^7 I-P
12/27/96	B-10	Cell	2×10^7 I-P
1/10/97	B-10	Cell	2×10^7 I-P.
1/23/97	B-10	Cell	1×10^7 I-P.
2/13/97	B-10	Cell	1×10^7 I-P.
2/20/97	B-10	Cell	2×10^7 I-P
3/6/97	PPD #2 + CFA	20mg I-P.	
4/10/97	PPD #2 + IFA	30mg I-P.	
~~11/6/97~~ ~~11/28/97~~	~~PPD #2~~	CCR26	DEF 3 2×10^7 Cell I-P. (mitomycin)
11/19/97	CCR 26	DEF 3	Mitomycin treated cell 2×10^7 I-P
12/4/97	CCR 26	DEF 3	Mitomycin treated cell 2×10^7 I-P.
12/19/97	CCR 26	DEF 3	Mitomycin treated cell 2×10^7 I-P.

Cell counts:

SC210

$1.58 \times 2 \times 10^4$ / ml 3 ml

9.36×10^7

Spleen cells

86×10^7 live cells

4.1×10^8

Fused as before — 2 wells HAT — 2 wells

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
	9-1-94	Recorded by	12/22/97

tabbies

EXHIBIT I

From Page No.

Tuesday, December 30th 1997

Staining of CCR2b. DEF3 subclone #60 with supernatants of fusion produced by Nasim on 12/22/97. Cells fixed and held overnight at 4°C.

Amy L. Reinhart 12/31/97

Wednesday, December 31st 1997

X → looks borderline ☐ → looks positive

✓ => accept (or underline)

plate 1 ~~F10~~ ~~E10~~ ~~D9~~ [D9] [F8] [F6] [G5]

plate 2 ~~D4~~ ~~H4~~ both on line

plate 3 H4✓ ~~H6~~ ~~E8~~

plate 4 1E (smear) 2E (two peaks) 2H (two peaks) 3E (smear) ~~3G~~
4E (two peaks) 4H (two peaks) 5E (smear) [6B] ←
~~6D~~ 7E (smear, two peaks) 8E etc 9-11E etc ~~12H~~ ✓

plate 5 2F✓ C8✓ both above line C8 is higher

plate 6 ~~A6~~ ~~6G~~✓ ~~7H~~ ~~8G~~ ~~8H~~

plate 7 E7 weak ~~F7~~ ~~H7~~ D8✓

plate 8 2F✓ 2G✓ 6A weak ~~8E~~ 9F✓ 11B

plate 9 ~~7A~~ 9F two peaks ~~10C~~✓ ~~10E~~✓

plate 10 1E two peaks 2F✓ ~~2G~~✓ ~~5G~~ 6C✓ ~~8C~~✓

Positives: 1D9 1F8 1F6 1G5 2D4 2H4 3H4 4B6 4H12
5F2 5C8 6G8 6H8 6G6 7D8 8F2 8G2 8F9
9C10 9F11 10F2 10C6 10C8

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
Suzanne Arenarae	3/20/98	Recorded by Amy L. Reinhart	12/30/97

EXHIBIT
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ACs Analysis

tants
fixed

ko positive
underline)

4H12
2 8F9

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TITLE Experiment #289/Fusion positives FACs Analysis Project No. Book No. 213

97

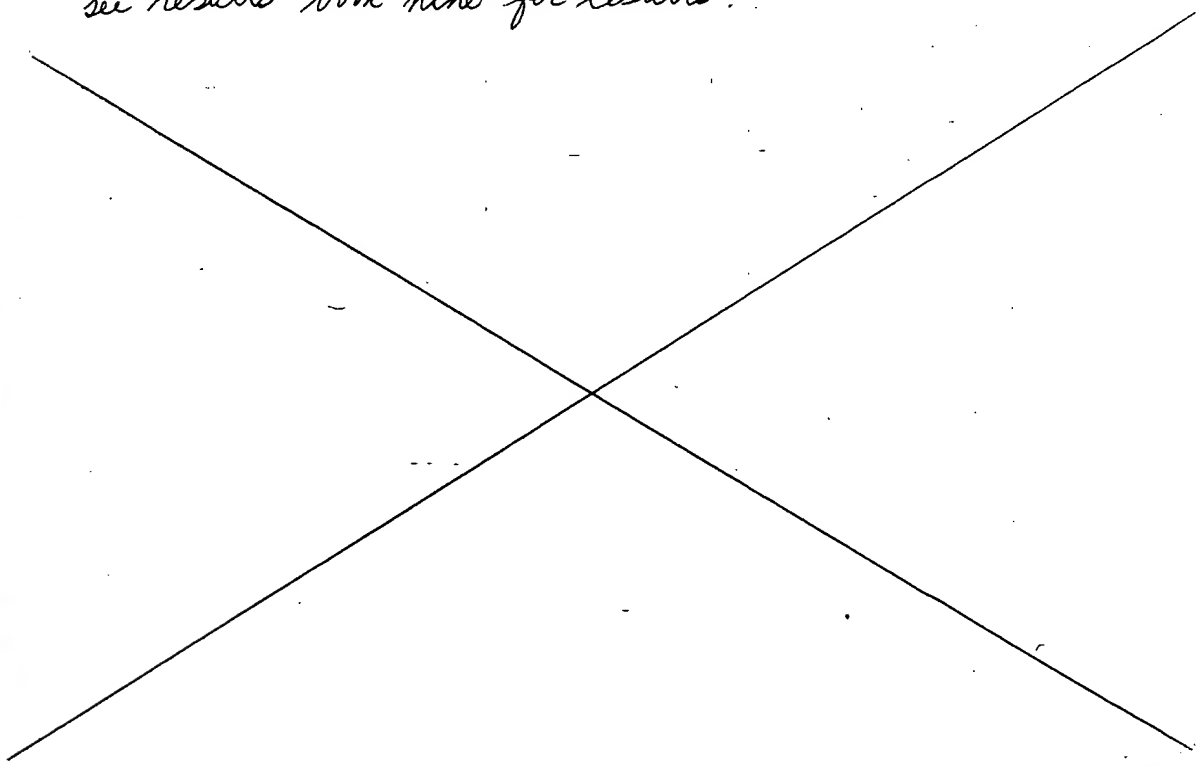
From Page No.

Friday, January 2nd 1998

Supernatants removed from clones of fusion that were determined to be positive and used as first antibody to stain CCR2b.DEF3 clone #60 and untransfected L1/2.

109 and 862 were both positive on CCR2 and negative on untransfected L1/2. All other clones were either negative on CCR2 or positive on L1/2.

See results book nine for results.

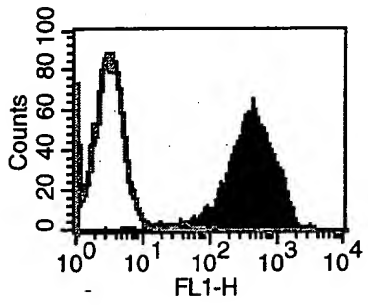


To Page No.

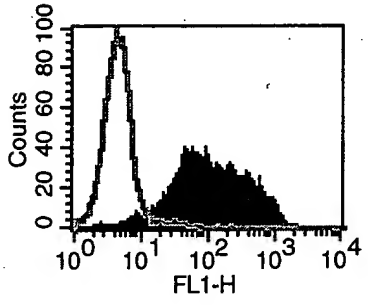
Witnessed & ~~Understood~~ by me,	Date	Invented by	Date
Suzanne W Desmarais	3/20/98	Recorded by Amy L. Reinhart	1/2/98

Fusion LS132
1/2/98
CCR2b

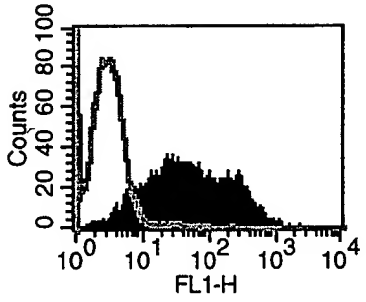
Staining of 1D9 on
L1/2 vs CCR2b.DEF3/60



Staining of 8G2 on
L1/2 vs CCR2b.DEF3/60



Positive Control: 5All



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Lab R c rd

Book number 213
Page number 97

EXHIBIT
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p. K-2
tabbies

From Page No.

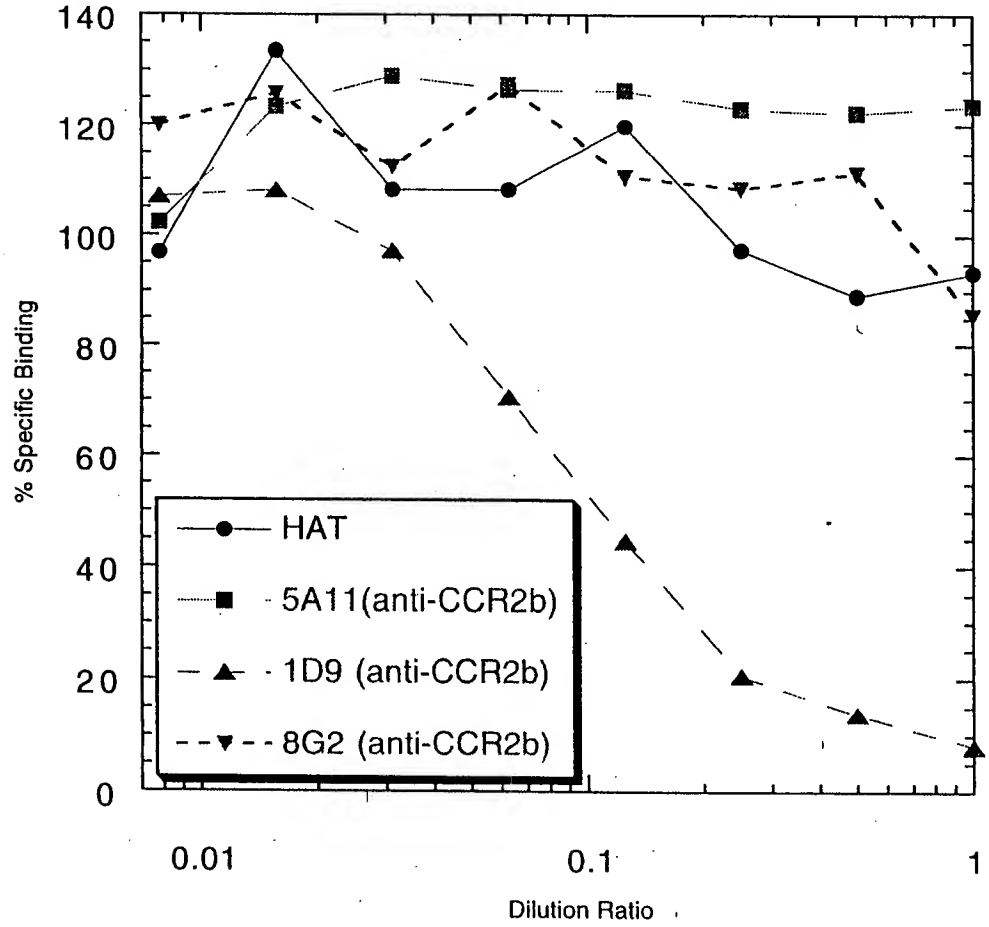
Friday January 9th 1998

Test of the ability of 1D9, 8G2 and a rat MCP-1 to block binding on THP-1 and CCR2DEF3 cells, HAT, PBS and 1C6 used as negative controls. 5A11 also tested.

See results book nine for protocol and results

Witnessed & Understood by me,	Date	Invented by	Date
Suzanne W Densmore	3/20/98	Recorded by Amy L. Reinhart	1/9/98

Binding #301
1/9/97 213/109
Antibody/Whole Cell Binding
CCR2DEF3.L1/2 Cells



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Lab Record

Book number 213
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